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LINKAGE ANALYSES OF SCHIZOPHRENIA TO CHROMOSOME 6p24-p22: AN ATTEMPT TO REPLICATE

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The present study evaluates evidence for linkage of schizophrenia to chromosome 6p24-p22. An independent sample of 211 families ascertained on the basis of having an affected sib-pair diagnosed with schizophrenia or schizoaffective disorder was assessed with seventeen polymorphic markers spanning a 37cM region. Linkage analysis was performed with parametric and non-parametric methods to test for cosegregation using 4 models of inheritance. Neither two-point nor multipoint non-parametric analyses reached significance at a level less than 0.01 for any markers examined in the region and lod score analyses were not suggestive of linkage. Based on initial findings in the present data set and recently published linkage results, two specific areas were densely covered with markers and tested for linkage disequilibrium. After correcting for multiple comparisons within each locus, no significant deviation from expected allele transmission ratios was observed. The present findings together with the published literature fail to find consistent evidence of a linkage for schizophrenia to a single locus on chromosome 6.

KEY WORDS: chromosome 6p, linkage, Schizoaffective Disorder, sib-pair analysis.

INTRODUCTION

Recent publications based on a large Irish cohort, have shown positive linkage of schizophrenia to a fairly large region on chromosome 6p (Wang et al., 1995; Straub et al., 1995). Lod scores varied, but peaked at 3.9 with marker D6D260 using a model that assumed heterogeneity and used broad diagnostic criteria that included non-affective psychoses and schizophrenia spectrum personality disorders as part of the phenotype (see Table I). Three independent groups have claimed to replicate this finding on the assumption that the region containing the putative gene covers at least 30cM (Antonarkis et al., 1995; Moises et al., 1995; Schwab et al., 1995). Others

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Table I: Review of previous reports examining a chromosome 6p24-22 linkage to schizophrenia. Abbreviations: Sz=schizophrenia, SA=schizoaffective, PNOS=psychosis not otherwise specified, Aff.=affecteds.

REFERENCE	#OF FAMILIES	LOCUS	MAX. LOD SCORE	THETA	MODEL
Straub et al. 1995	265	D6S296*	3.51	0.004	Broad: All psychoses plus related spectrum Dx Pen genetic model
Wang et al. 1995	186	D6S260	3.9	0.175	Partially Dominant Heterogeneity allowed
Antonarakis et al. 1995	57	D6S296**	1.17	0.25	Recessive, aff. Only Aff.=Sz, SA only No heterogeneity
Gurling et al. 1995	23	D6S285	0.21	0.30	Aff.=Sz, SA, PNOS Recessive Heterogeneity allowed
Moises et al. 1995	65	D6S274	Non-Parametric Analyses only p=0.005		Aff.=Sz, SA only
Mowry et al. 1995	45	D6S285	0.37	0.00	Aff.=Sz, SA other nonaffective psychoses Recessive, reduced penetrance Heterogeneity allowed
Schwab et al. 1995	54	D6S285***	2.00	0.00	Aff.=Sz or SA Affecteds only Additive model

* sib-pair analysis: D6S296 max. P=0.03; D6S285 max. P=0.005
** sib-pair analysis: D6S296 p=0.004
*** sib-pair anlyasis: D6S470 p<0.004; D6S260 p<0.033; D6S274 p<0.007 D6S285 p<0.003; D6S461 p=0.002; D6S258 p=0.001;

have failed to find linkage within this region (Gurling et al., 1995; Mowry et al., 1995), although it can be argued that with an assumption of heterogeneity and an estimate of a maximum of 30% families linked, negative findings will occur if the sample size is small.

The following is a report of results in this region for a large cohort of families (N=211), most of whom have at least one set of sibling pairs suffering from schizophrenia.

METHODS

Clinical

Three international sites have been used as bases for the recruitment of families with at least two available siblings diagnosed with schizophrenia or schizoaffective disorder. Identification of families, clinical evaluative and diagnostic procedures were similar in all locations. Recruitment was done using several methods: catchment area screening, systematic contact with health professionals at hospital and outpatient facilities within one day's driving time from the center, and advertisement through local and national support organizations for families of the mentally ill (i.e. The National Alliance for Mental Illness, NAMI, in the USA; Schizophrenia A National Emergency, SANE in the UK). Thus, for the present chromosome 6 laboratory analysis, 146 families from the USA, 35 from the UK and Ireland, 28 from Northern Italy, and 2 from Belgium were examined (total = 211). All subjects signed written informed consent for participation in these studies.

Diagnoses were made using DSM-III-R criteria, based on a combination of a structured modified SADS interview (Schedule for Affective Disorder and Schizophrenia, Spitzer and Endicott, 1978) combined with the SIDP (Structured Interview for Personality Disorders, Pfohl, 1990) or the DIGS (Diagnostic Interview for Genetic Studies, Nurnberger et al., 1994), a structured questionnaire asked of reliable family informants about other family members, and medical records as indicated. The physicians and other professionals performing clinical evaluations (approximately 2 individuals per site) have been trained in these procedures by one of us (LED) and all have undergone periodic diagnostic reliability exercises to maintain consistency between centers. Final diagnoses were made by consensus in the UK by J Loftus, L DeLisi and T Crow, Belgium by M DeHert and L DeLisi, Italy by M Comazzi and A Vita, and the USA by L DeLisi, G Shields and M Kushner. All the above individuals have undergone periodic diagnostic reliability exercises with kappa statistic scores ranging between 0.85 and 0.90 for primary diagnoses. Dr. DeLisi travels to each site periodically for reviewing diagnoses and supervising procedures. Initially all interviewers spend several days at the Stony Brook site to train in all methods used.

Pedigrees for each family were diagrammed and all families received research code numbers and separate numbers for each individual. Computer diagnostic files were maintained without knowledge of laboratory marker data. Similarly, laboratory marker data were entered into the file without knowledge of diagnostic information. The families, predominantly nuclear, consisted of 215 affected sibling pairs with either schizophrenia or schizoaffective disorder. 102 families had both parents genotyped, while 84 had one parent genotyped.

Laboratory

Radiation hybrid mapping. A radiation hybrid panel (Cox et al., 1990) was assayed by PCR and scored for 18 chromosome 6 markers. The results were sent to the Stanford RH server (e-mail address: rhserver@shgc.stanford.edu) and two-point linkage analysis data were returned which showed evidence for 5 linkage groups. Markers within the linkage groups were then typed on the lower resolution Genebridge 4 panel (Walter et al., 1994). Two-point linkage analysis was performed using RHMAX2PT to link these groups (Boehnke et al., 1991). Multipoint analysis using RHMAXLINK was performed on markers making up distinct linkage groups to get the most likely local order. Distances between the markers are expressed in cR. For the G3 panel, $1cR_{8000}$ is equivalent to approximately 30kb and for the Genebridge 4 panel, 1 cR_{3000} equals approximately 270kb. The order of these loci by RH mapping reported here is consistent with the Whitehead/MIT YAC-based STS-content and RH maps of the region, with minor differences in local order in one of the linkage groups. This is probably due to the fact that this order was established using a higher resolution RH panel than that used by the Whitehead/MIT for their map and these markers are relatively close together (within 1.3Mb). The marker order is also consistent with the genetic map reported here (figure 1).

Genotyping. DNA was prepared from stored lymphoblastoid cell lines or fresh blood. PCR primer pairs for the seventeen markers were synthesized according to published sequences flanking polymorphic repeats. DNA amplification was performed using the PTC100 thermocycler (MJ Research, San Francisco, CA). The forward primer of each pair was labelled with one of the three fluorescent dyes, FAM, HEX, or TET, (applied Biosystems, Foster City, CA) to enable detection. PCR was carried out in a final reaction volume of twenty microliters. Amplification occurred during 35 cycles each of 94 degrees C for 30 seconds, 30 seconds at the primer specific annealing temperature, followed by 30 seconds at 72 degrees C. PCR products were subjected to electrophoresis on an

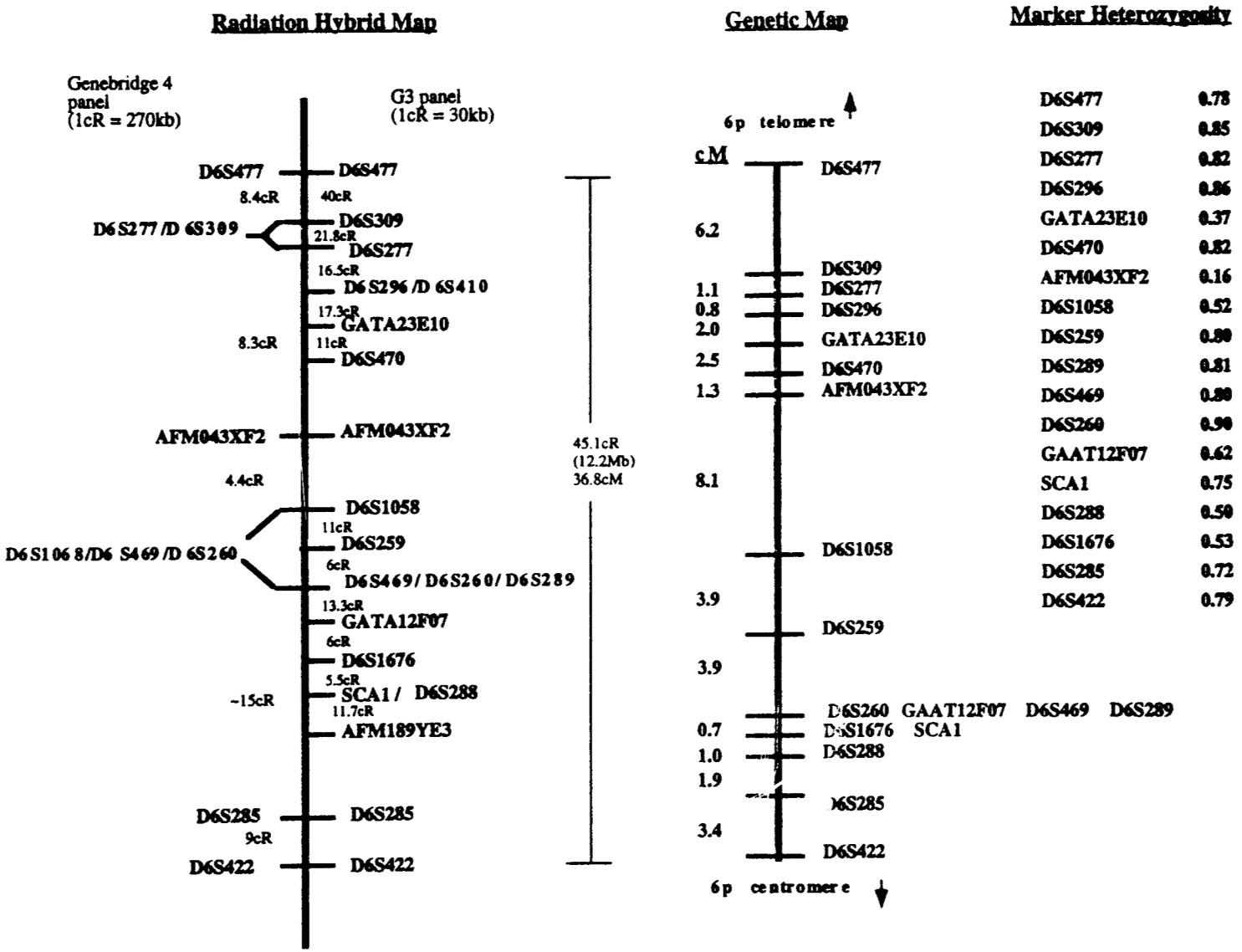

Fig. 1. Radiation hybrid and genetic maps of markers analyzed in 6p region. For the radiation hybrid map, $1cR_{8000}$ is approximately equal to 30 kb using the G3 panel, and 1 $c3_{3000}$ is approximately equal to 270 kb using the Genebridge 4 panel. Marker heterozygosities were calculated using observed allele frequencies from the present sample.

ABI373a, using a 12cM well-to-read denaturing 6% polyacrylamide gel, with analysis using Applied Biosystems' GeneScan 1.2. Not all individuals with DNA available had types available for all markers due to technical problems identifying occasional genotypes. Genotypes per family ranged from 2-16. A total of 13,623 genotypes were successfully typed for this study.

Data Analyses. Two-point affecteds only linkage analyses were conducted for three diagnostic categories: (1) Chronic Schizophrenia only and (2) Schizophrenia plus Schizoaffective Disorder, and (3) A "Broad Diagnosis", including schizophrenia, schizoaffective, other non-affective psychosis, schizotypal or paranoid personality disorders. Two-point lod scores were computed using the program MLINK of FASTLINK (Cottingham et al., 1993) with recombination fractions varied from 0.0 to 0.5 at intervals of 0.02. Genetic heterogeneity was assessed using the program HOMOG (Ott, 1991). Four genetic models for two narrow diagnostic criteria were taken directly from Straub et al. (1995) as seen in Table II. The penetrance values and disease allele frequencies were originally calculated based on segregation analyses using the pedigrees collected in Ireland. The marker allele frequencies used were calculated directly from the observed genotype data in the present study; no appreciable differences were seen in comparison with published frequencies in public databases.

Non-parametric analyses were carried out using the three definitions of affected status (chronic schizophrenia only, schizophrenia plus schizoaffective disorder, and the "Broad" category). The proportion of (IBD) in affected sib-pairs was tested against a null hypothesis of no linkage alleles shared Identity by Descent (0.50). To account for sib-pairs of missing or uninformative parents, the methods of Haseman and Elston (1972) was used to estimate proportions of alleles shared IBD. The observed marker allele frequencies were used in this procedure. The affected sib-pair analysis was carried out using the SIBPAL program of the SAGE package (Tran et al., 1994). Multipoint linkage analysis of the affected sib pairs was conducted using MAPMAKER/SIBS computer program (Lander and Kruglyak, 1995). In these analyses, dominance variance was assumed to be negligible and all pairings of siblings within a sibship were analyzed as if independent.

Maximum lod scores were derived from maximum likelihood 2-1-0 IBD sharing as described (Holmans, 1993). Tests for linkage disequilibrium were conducted using the Transmission /Disequilibrium Test (Spielman et al., 1993). Chi-square analyses were performed on the 4 most common alleles because the sample size was too small to draw conclusions on rarer alleles and this also reduced the number of statistical tests performed.

RESULTS

Marker Information and Map of the Region

Figure 1 shows the marker information with both the physical and genetic maps of the region. The map consists of 18 markers spanning 36.8cM genotyped on 211 families. The genetic map and marker heterozygosities were

Table II:

Genetic Model	Penetrance			Disease Allele Frequency
	DD	Dd	dd	
Dominant	0.55	0.55	0.0006	0.0049
Penetrance	0.55	0.275	0.0006	0.0098
Additive-liability	0.55	0.061	0.0007	0.0378
Recessive	0.55	0.0006	0.0006	0.0991

calculated directly from the observed genotypes. A radiation hybrid map was constructed to confirm the genetic map marker ordering. The relative order and spacing of the markers by RH mapping is consistent with the published physical map of the region. A gap of 8.1 cM separates the distal and proximal regions selected for the highest density marker saturation. Marker AFM043XF2 was excluded from the analysis due to its low observed heterozygosity.

Parametric Linkage Analysis

Three separate linkage analyses were undertaken for each of the genetic and diagnostic models described above. Tables IIA, B and C show maximal lod scores, corresponding theta values and estimates of the proportions of families linked to each locus (alpha). No marker showed significant evidence for linkage using the threshold Zmax > 3.0 for any diagnostic category. Only the marker D6S422 revealed a lod score greater than 1.0. This result was observed for the chronic schizophrenia only category under the dominant and penetrance models of Straub et al. (1995). This marker is located at the centromeric end of the region studied.

No other lod scores provided any evidence of linkage.

Non-parametric Analyses

The results of the mean allele-sharing analysis (SIBPAL) on 17 markers are presented in Table IV. Minimal evidence for linkage to schizophrenia is seen at marker D6S470 (nominal p=0.01) but disappears with broader diagnoses included. Nearby markers do not show evidence for linkage. No corrections of the significance levels were made for the multiple tests conducted in these analyses.

Results of the multipoint sib-pair analyses are shown in Figure 2. The greatest maximum lod score was obtained for the chronic schizophrenia phenotype in the telomeric region, spanning markers D6S309 to D6S470 (MLS>1.0<1.1). Neither of the other diagnostic classifications exceeded 0.30, indicating no evidence for linkage to these phenotypes. In these families, there are very few individuals meeting the broad diagnosis criteria who do not meet the schizophrenia/schizoaffective disorder criteria. Thus, results for the two phenotypes reveal a similar pattern of effects.

Exclusion MLS scores for the same three diagnostic categories are shown in figures 3A and 3B. These results were obtained by evaluating MLS scores under the condition of a single locus (lambda s = 1.2, 2, 4, and 6). The outcomes for chronic schizophrenia, shown in figure 3, indicate that a schizophrenia locus having relatively small effect cannot be excluded from the region (lambda s = 1.2 and 2.0), but a major gene having lambda s > 4.0 can be excluded from the entire region with lod < -2.0. The centromeric markers show the strongest evidence against a major gene effect, revealing exclusion scores <-5.0 centered around the SCA1 marker. Exclusion scores for schizophrenia/schizoaffective disorder and for the broad diagnosis category, shown in Figure 3B, indicate that the entire region can be excluded for a locus as small as lambda s = 2.0.

Linkage Disequilibrium Analysis

Due to preliminary results seen in the present data set (Lichter, 1995) and published linkage reports the number of transmissions and non-transmissions of the four most common alleles at each locus were compared to the expected 1:1 ratio by chi-square tests. A significant deviation from the expected ratio of transmission, as assessed by chi-square tests, was taken as evidence for linkage disequilibrium. All 17 markers were analyzed under all three of our diagnostic categories, the strongest result was obtained for marker D6S277 (chi-square = 8.56, p=0.01) with a chronic schizophrenia only diagnosis. This marker is located

Table IIIa: Two-point lod scores for chromosome 6p: chronic schizophrenia[a].

	Dominant model			Penetrance model			Additive-liability model			Recessive model		
Marker	Z	θ	α	Z	θ	α	Z	θ	α	Z	θ	α
D6S477	0.157	0.30	1.00	0.164	0.28	1.00	0.174	0.28	0.94	0.235	0.30	0.99
D6S309	0.444	0.00	0.23	0.436	0.00	0.24	0.172	0.18	0.34	0.260	0.32	0.98
D6S277	0.418	0.28	1.00	0.412	0.28	1.00	0.243	0.30	0.98	0.410	0.30	0.96
D6S296	0.268	0.00	0.15	0.250	0.00	0.15	0.216	0.00	0.14	0.378	0.00	0.13
GATA23E10	0.735	0.16	1.00	0.706	0.16	1.00	0.478	0.18	0.97	0.481	0.08	0.41
D6S470	0.842	0.24	1.00	0.871	0.22	0.97	0.943	0.24	1.00	0.780	0.28	0.99
D6S1058	0.000	0.50	N/A	0.000	0.50	N/A	0.000	0.50	N/A	0.000	0.50	N/A
D6S259	0.319	0.30	1.00	0.266	0.30	1.00	0.065	0.00	0.07	0.056	0.00	0.04
D6S469	0.506	0.22	0.71	0.486	0.22	0.74	0.269	0.30	1.00	0.176	0.34	0.97
D6S260	0.227	0.00	0.13	0.195	0.00	0.13	0.004	0.00	0.02	0.015	0.00	0.02
D6S289	0.136	0.36	1.00	0.114	0.36	1.00	0.001	0.00	0.01	0.026	0.00	0.03
GAAT12F07	0.019	0.36	0.75	0.008	0.34	0.39	0.000	0.50	N/A	0.000	0.50	N/A
D6S1676	0.041	0.00	0.09	0.032	0.00	0.08	0.000	0.50	N/A	0.000	0.50	N/A
SCA1	0.184	0.32	1.00	0.138	0.34	1.00	0.000	0.50	N/A	0.000	0.50	N/A
D6S288	0.109	0.32	1.00	0.084	0.34	1.00	0.001	0.00	0.01	0.001	0.000	0.01
D6S285	0.389	0.26	1.00	0.370	0.26	1.00	0.160	0.32	1.00	0.157	0.340	1.00
D6S422	1.065	0.20	1.00	1.021	0.20	1.00	0.445	0.26	1.00	0.317	0.320	1.00

[a]Z refers to maximum lod score under heterogeneity, θ indicates the corresponding recombination fraction, and α represents the proportion of linked families. Parameters of the four models are identical to those used by Straub et al. (1995).

Table IIIb: Two-point lod scores for chromosome 6p: schizophrenia + schizoaffective disorder[a].

	Dominant model			Penetrance model			Additive-liability model			Recessive model		
Marker	Z	θ	α	Z	θ	α	Z	θ	α	Z	θ	α
D6S477	0.136	0.34	1.00	0.129	0.34	1.00	0.142	0.30	0.73	0.153	0.34	0.92
D6S309	0.116	0.42	1.00	0.096	0.42	1.00	0.027	0.44	1.00	0.022	0.46	1.00
D6S277	0.116	0.38	1.00	0.092	0.38	1.00	0.030	0.22	0.12	0.038	0.28	0.16
D6S296	0.036	0.42	1.00	0.024	0.42	1.00	0.093	0.00	0.06	0.197	0.00	0.06
GATA23E10	0.080	0.36	1.00	0.052	0.38	1.00	0.001	0.46	1.00	0.000	0.50	N/A
D6S470	0.212	0.36	1.00	0.206	0.36	1.00	0.385	0.00	0.10	0.291	0.00	0.06
D6S1058	0.436	0.22	0.95	0.459	0.22	1.00	0.416	0.00	0.28	0.329	0.18	0.45
D6S259	0.391	0.32	1.00	0.310	0.32	1.00	0.010	0.00	0.02	0.021	0.00	0.02
D6S469	0.833	0.10	0.30	0.748	0.10	0.31	0.438	0.30	0.99	0.420	0.00	0.11
D6S260	0.470	0.34	1.00	0.404	0.34	1.00	0.157	0.38	1.00	0.238	0.00	0.07
D6S289	0.351	0.34	1.00	0.302	0.34	1.00	0.090	0.40	1.00	0.071	0.00	0.04
GAAT12F07	0.362	0.30	1.00	0.256	0.30	1.00	0.012	0.38	0.59	0.000	0.50	N/A
D6S1676	0.016	0.40	1.00	0.009	0.42	1.00	0.000	0.50	N/A	0.000	0.50	N/A
SCA1	0.626	0.00	0.18	0.489	0.00	0.17	0.102	0.00	0.07	0.041	0.00	0.04
D6S288	0.053	0.40	1.00	0.018	0.44	1.00	0.000	0.50	N/A	0.000	0.50	N/A
D6S285	0.827	0.00	0.19	0.663	0.00	0.19	0.341	0.32	1.00	0.256	0.36	1.00
D6S422	0.776	0.00	0.17	0.608	0.00	0.17	0.305	0.00	0.13	0.242	0.00	0.09

[a]Z refers to maximum lod score under heterogeneity, θ indicates the corresponding recombination fraction, and α represents the proportion of linked families. Parameters of the four models are identical to those used by Straub et al. (1995).

Table IIIc: Two-point lod scores for chromosome 6p: broad diagnosis[a].

	Dominant model			Penetrance model			Additive-liability model			Recessive model		
Marker	Z	θ	α	Z	θ	α	Z	θ	α	Z	θ	α
D6S477	0.165	0.34	1.00	0.164	0.32	0.98	0.081	0.22	0.27	0.067	0.18	0.14
D6S309	0.020	0.46	1.00	0.001	0.46	1.00	0.000	0.50	N/A	0.000	0.50	N/A
D6S277	0.110	0.38	1.00	0.098	0.38	1.00	0.066	0.16	0.12	0.124	0.24	0.22
D6S296	0.052	0.40	1.00	0.055	0.00	0.05	0.173	0.00	0.08	0.359	0.00	0.09
GATA23E10	0.176	0.32	1.00	0.132	0.32	1.00	0.000	0.50	N/A	0.000	0.50	N/A
D6S470	0.046	0.42	1.00	0.029	0.42	1.00	0.243	0.00	0.07	0.253	0.00	0.06
D6S1058	0.725	0.20	1.00	0.754	0.18	0.97	0.590	0.04	0.40	0.406	0.16	0.44
D6S259	0.756	0.28	1.00	0.616	0.28	0.99	0.014	0.00	0.03	0.045	0.00	0.03
D6S469	0.737	0.20	0.50	0.697	0.22	0.61	0.52€	0.28	0.92	0.643	0.02	0.16
D6S260	0.351	0.34	1.00	0.279	0.34	1.00	0.051	0.38	0.81	0.145	0.00	0.06
D6S289	0.136	0.38	1.00	0.089	0.38	1.00	0.000	0.50	N/A	0.051	0.00	0.04
GAAT12F07	0.340	0.30	1.00	0.254	0.30	0.97	0.032	0.36	0.69	0.014	0.38	0.50
D6S1676	0.059	0.36	0.99	0.043	0.38	1.00	0.000	0.50	N/A	0.000	0.50	N/A
SCA1	0.625	0.28	0.97	0.488	0.28	0.93	0.096	0.00	0.07	0.059	0.00	0.04
D6S288	0.073	0.38	1.00	0.024	0.42	1.00	0.000	0.50	N/A	0.000	0.50	N/A
D6S285	0.907	0.00	0.20	0.707	0.00	0.20	0.326	0.00	0.13	0.208	0.00	0.08
D6S422	0.714	0.00	0.15	0.567	0.00	0.15	0.423	0.00	0.15	0.482	0.00	0.13

[a]Z refers to maximum lod score under heterogeneity, θ indicates the corresponding recombination fraction, and α represents the proportion of linked families. Parameters of the four models are identical to those used by Straub et al. (1995).

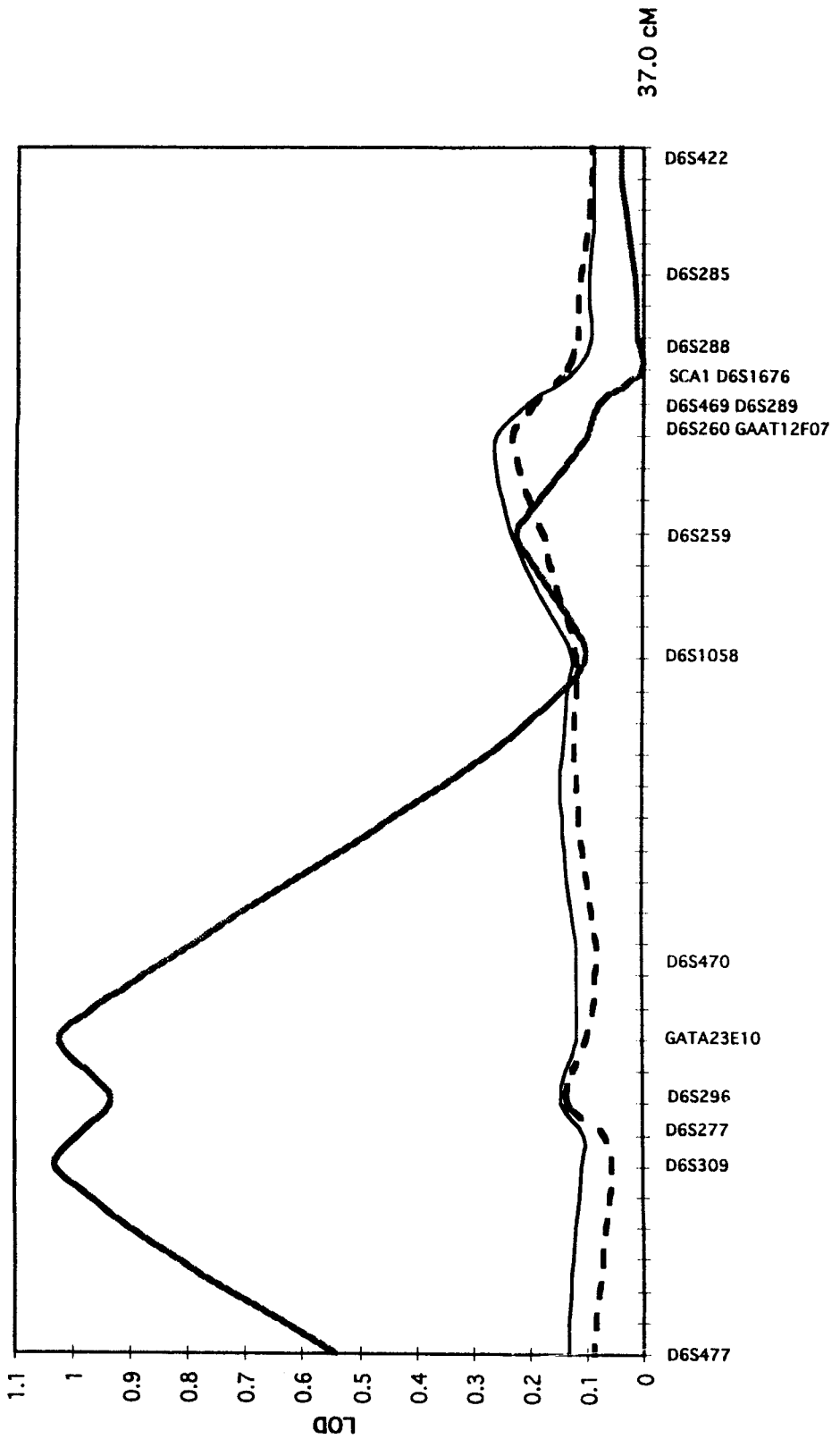

Figure 2: Multipoint maximum lod score curves for chronic schizophrenia (thick line), schizophrenia + schizoaffective disorder (long dashed line), and broad diagnosis (short dashed line). Marker distances correspond to those shown in Figure 1.

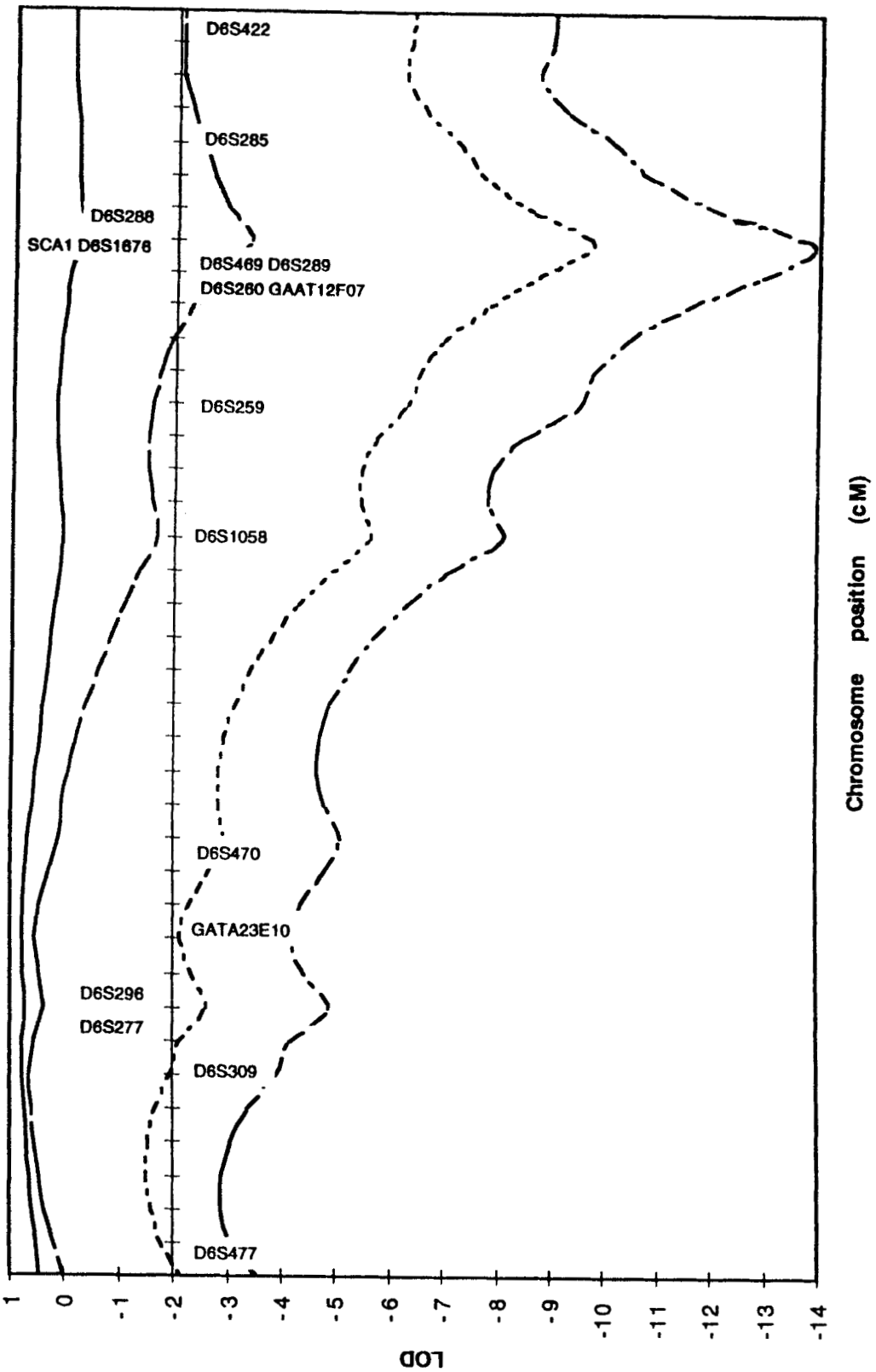

Fig. 3A: Exclusion map for chronic schizophrenia. Values of s analyzed include: 1.2 (continuous line); 2.0 (long dashed line); 4.0 (short dashed line); 6.0 (broken dashed line).

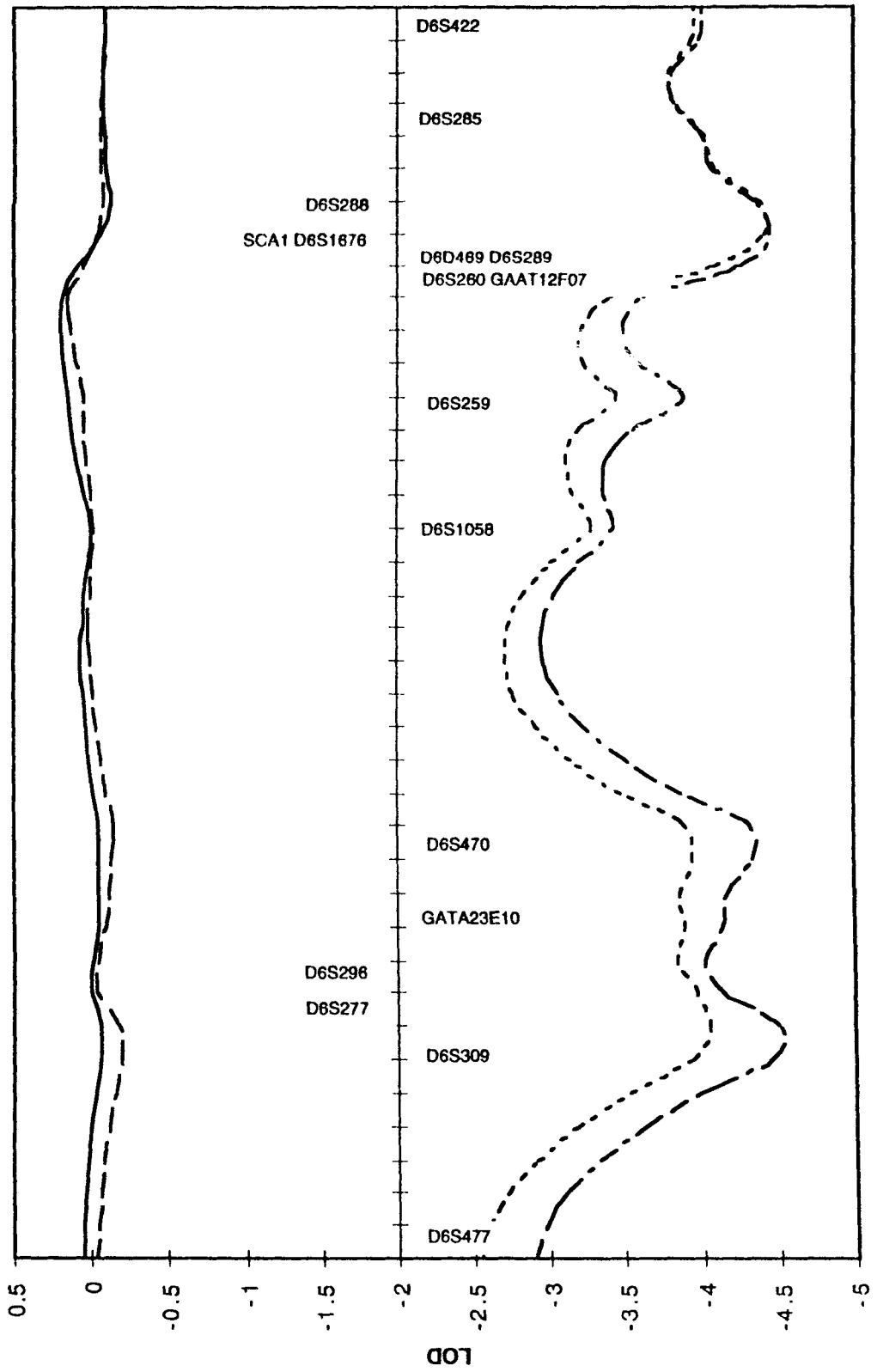

Fig. 3B: Exclusion map for schizophrenia + schizoaffective disorder and the broad diagnosis. Values of ls and the corresponding diagnostic criteria are: s = 1.2, schizophrenia + schizoaffective disorder (continuous line); s = 1.2, broad diagnosis (long dashed line); s = 2.0, schizophrenia + schizoaffective disorder (short dashed line); s = 2.0, broad diagnosis (broken dashed line).

Table IV: Sib pair analyses for each diagnostic model.

	DIAGNOSES					
	Schizophrenia (N=124)		Schizophrenia + Schizoaffective (N=215)		Broad Diagnosis (N=217)	
Marker:	pi	p	pi	p	pi	p
D6S477	0.53	0.15	0.53	0.12	0.53	0.14
D6S309	0.53	0.20	0.49	1.00	0.49	1.00
D6S277	0.55	0.05	0.53	0.13	0.53	0.13
D6S296	0.53	0.18	0.51	0.29	0.52	0.26
GATA23E10	0.52	0.16	0.50	1.00	0.50	1.00
D6S470	0.56	0.01	0.53	0.11	0.52	0.16
D6S1058	0.51	0.39	0.52	0.10	0.52	0.07
D6S259	0.51	0.39	0.51	0.38	0.51	0.38
D6S289	0.50	0.46	0.51	0.37	0.51	0.37
D6S469	0.53	0.18	0.54	0.06	0.54	0.04
D6S260	0.50	0.46	0.52	0.20	0.52	0.18
GAAT12F07	0.49	1.00	0.52	0.20	0.52	0.20
D6S1676	0.49	1.00	0.49	1.00	0.49	1.00
SCA1	0.50	0.46	0.53	0.10	0.53	0.10
D6S288	0.50	1.00	0.50	1.00	0.50	0.48
D6S285	0.53	0.08	0.52	0.10	0.53	0.08
D6S422	0.54	0.09	0.52	0.14	0.53	0.12

within the only area which showed possible evidence for linkage in the nonparametric multipoint analyses (figure 2). However, with a further correction for number of markers run, this failed to reach significance. No other markers showed suggestive linkage disequilibrium at a p value of <0.01.

DISCUSSION

The present study was an attempt to confirm the linkage findings reported by Straub and colleagues (1995) using a large cohort of affected sibling pairs and multiple methods of analyses, including the models used in the above publication. The cohort assembled here is larger than that reported in Wang et al. (1995) and comparative although a little smaller in numbers to that in Straub et al. (1995). Two specific regions, showing suggestive evidence of linkage in previous studies (Table I) spanning a 30cM region of chromosome 6p, were tested with a high degree of marker density (approximately 1 marker/cM). Both non-parametric sib-pair tests and the TDT showed some markers with borderline significance at p values less than 0.05, but greater than 0.01; however, lod scores using published models failed to provide evidence of linkage. One possible explanation for this outcome is that the estimate of 15-30% linked families in the Irish sample may be an over estimate for our population. If the proportion of linked families is considerably less, then linkage will be more difficult to detect.

Of course, an alternative explanation is that the previous published reports were false positive findings, perhaps due to inflation of lod scores through multiple testing, phenotype ambiguities, use of a range of recombination frequencies, or assumption that positivity by non-parametric methods is enough to conclude that a positive linkage is present (Baron, 1996; Kendler et al., 1996).

Analyses in the present study were first carried out with the most conservative definition of affected

status, chronic schizophrenia only, as an attempt to define a more homogeneous diagnostic group. Although family risk studies have shown that schizophrenia and schizoaffective disorder are related, recent evidence from the Roscommon Irish family study suggests that the diagnosis of DSM-III-R schizoaffective disorder defines a syndrome that differs from both schizophrenia and affective disorder, perhaps resulting from a high genetic liability to both illnesses (Kendler et al., 1995). However, taken together, our nonparametric and parametric, as well as linkage disequilibrium analyses, do not support this distinction in the chromosome 6p region by delineating a consistently linked region for one of these diagnostic models.

In summary, evidence for linkage in this study does not read statistical significance, although the results appear most positive in the vicinity of markers D6S309-D6S470 where two previous independent linkages of varying degrees of positivity have been found (Straub et al., 1995; Antonarakis et al., 1995). However, in our families the strongest linkage is to the narrow diagnostic model, chronic schizophrenia alone. Unlike the previous published data, a broader classification including individuals with schizoaffective disorder and other schizophrenia-spectrum diagnoses resulted in a loss of observable effect. Moreover, when all the linkage analysis reports on this region are considered together and the whole 30cM region of chromosome 6p24-p22 is examined, despite varying degrees of positivity at different sites (Wang et al., 1995; Moises et al., 1995; Schwab et al., 1995), no consistent support for a single locus emerges. Thus, if a gene for psychosis exists within this region, it is likely to account for illness in only a small number of families, or be one amongst a few genes of minor to moderate effect that interact to cause the illness. If the latter is the case it may take a considerably larger number of families to replicate the original finding.

Further investigation in the current sample of 211 families is not continuing in this region of chromosome 6p, at least until similar analyses have been performed throughout the entire genome in the same families for comparison. These studies are now in progress.

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